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Foreword

Over the last five years, the East, Central and Southern African Health Community (ECSA-HC) has continued to undertake advocacy and technical assistance to assist member countries to embrace and scale up food fortification initiatives as a key strategy to reduce micronutrient malnutrition in the region.

ECSA has been working with partners in direct response to resolutions of the Conference of Health Ministers to scale up food fortification initiatives as a critical strategy in fighting the devastating effects of micronutrient malnutrition among populations of member states. ECSA partners in the Regional Food Fortification Initiative include the A2Z Project, USAID, UNICEF, Micronutrient Initiative (MI), and ICCIDD, among others.

Part of the outcome of the intensified collaborative initiative, is a series of fortification guidelines developed to guide the industry during the fortification process of staple foods and provide government food inspectors a reference point in enforcing the standards.

In order to ensure compliance with the set standards, a manual on laboratory tests for fortified foods was developed. This manual provides agreed upon test methods that have been tested over the years and provided reliable results in the analysis of micronutrients in fortified foods, within the region.

This manual is Part 1 of the Manual for Laboratory Methods for Fortified Foods. It specifies test methods for determination of iodine in iodated salt..

It is our hope that the use of this manual will help strengthen food control activities in our countries in order to deliver safe and quality fortified foods to the ECSA population.

Steven Shongwe
Executive Secretary
ECSA Health Community
Acknowledgement

This Manual has been developed by the ECSA Health Community Secretariat with technical and financial assistance from the Academy for Educational Development through A2Z: The USAID Micronutrient and Child Blindness Project (GHS-A-00-05-00012).

The manual is as a result of joint work by distinguished food fortification experts in developing countries. During the drafting of this manual, consultations with senior officers from food control departments of the ECSA member states were made and input incorporated.

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ECSA is deeply thankful to the above authors for preparing this manual.

Disclaimer

_The content of this manual can be adapted to suit country specific contexts. In such a case, the content of the resulting document will be the sole responsibility of the organization adapting the manual and will not represent the views of the authors and that of the ECSA-HC. The Use of the content of this manual should be duly acknowledged._

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INTRODUCTION

MANUAL OF LABORATORY METHODS FOR FORTIFIED FOODS

The ability to rapidly test for added micronutrients in fortified foods allows program managers to readily determine if the fortified food complies with the technical specifications, and it is an objective measurement of the program performance. It is therefore necessary to have easy access to laboratories which can determine the presence and content of common indicator micronutrients such as vitamin A, iron, riboflavin and iodine. The results that are generated from such laboratories provide vital information for establishing if: i. the fortification process at the factory level is working properly and micronutrient levels are within specified requirements, based on sampling and testing by the Quality Assurance and Quality Control Department, and on results from inspection and enforcement activities of the Food Control Unit of the government; ii. the fortified foods are reaching the retail stores with the expected conditions of fortification; and iii. imported fortified foods contain the micronutrient levels required in the national regulations and standards.

Ideally, all methods used for testing micronutrients should be selective, sensitive, accurate, precise, fast, and simple and have a low cost. In practice, the performance, complexity and cost of the methods will depend on several factors such as: the nature of the matrix (i.e. sugar or salt vs. wheat flour), the fortification compound used to fortify (i.e. reduced iron vs. ferrous salts), the available methods for detecting the analyte or micronutrient and the type of parameter recorded for quantification (i.e. titration volume based on change in color vs. absorbance readings for UV/Vis spectrophotometry). It is difficult to have single methods that fulfill all the characteristics mentioned above and so several methods have been developed for use at different levels of food enforcement, accordingly to the specific conditions and needs. The methods provided in this manual have proved to be cost-effective and adequate for the purposes outlined above. The methods are present in three categories namely qualitative, semi-quantitative and quantitative methods.

Qualitative methods: These are used to determine the presence of a nutrient and are ideal for screening samples to determine if the samples are fortified with the indicator micronutrient. Qualitative methods are usually simple, fast and cheap. These methods may also be used to determine samples which contain the indicator nutrients around a cut-off point in order to estimate percent of fortified food. Where possible, the initial screening of samples earmarked for quantitative tests using qualitative methods is beneficial. It helps in reducing the time and resources wasted in performing complicated and expensive quantitative tests on samples that are presumed fortified but do not contain the nutrient of interest.

Semi-quantitative methods: These methods are mainly used to monitor the micronutrient levels in the finished product during the fortification process at the factory. These methods are based on their respective qualitative methods, but are adapted to introduce comparative assessment based on intensity of color development or spot density. Quantification is based on comparing color intensity or spot density of
fortified samples against standard samples with known amounts of micronutrients. The results are reported as a range of values because of the uncertainties related to the determination of color intensity and spot density by analysts. Despite of this limitation, semi-quantitative methods help to determine whether the micronutrients added are within a specific acceptable range as determined by the local standards. The advantage of these methods is that they are fast, simple, cheap, and provide concentrations which, together with other factory parameters as amount of premix used per quantity of food produced, can be used for making timely decisions if problems are found. Results obtained using these methods have to be confirmed periodically using quantitative methods, either in the factory laboratory or by sending samples to external laboratories.

**Quantitative methods:** These methods are meant to accurately determine the concentration of micronutrients in the food. Results from quantitative testing of samples taken by the Food Control Units during inspection activities are crucial for determining whether a factory, distributor or brand is complying with the standards and regulations. Because the concentration levels determined during inspection have legal implications, quantitative, accurate and precise results are a must. These methods are also applicable to the quality control section in factories for verification purposes, and quantitative results are used to justify decisions regarding inspection activities at production centers, importation sites and retail stores. Most quantitative methods for micronutrients are time consuming, need special equipment and skillful and trained technicians, and as a result they are expensive. The only exception is the quantitative test for iodate in salt which is relatively less involving and low cost.

This manual presents analytical methods that have been used in the food fortification programs for several years. The spot test for iron in wheat flour, the methods for determining vitamin A in sugar and oil, and the method for determining iodate/iodine in salt are applied worldwide in the fortification programs. This manual also includes the determination of riboflavin in flours, as an indicator for the vitamins. Other methods have been applied only in some countries, and thorough validation is still needed. This is the case of the method to determine iron from ferrous sulfate in wheat flour.

1 Although iron is an effective indicator of choice for flour fortification, it is important to confirm compliance of the fortification formula in terms of vitamin content. Vitamin A is usually a vitamin of choice to complement iron determination. However, in cases where vitamin A is not added to the flours, riboflavin is a good alternative.

The following sections are included in the manual:

- Definitions and general description of the analytical methods
- Methods for determining iodine in salt
(A). DEFINITIONS OF ASSAY PARAMETERS AND GENERAL DESCRIPTION OF THE ANALYTICAL METHODS

I. ASSAY PARAMETERS

In this manual, the following definitions are used to characterize the performance of the analytical assays:

**Specificity** is the ability of a method to respond exclusively to the target analyte and not to any degrading impurity, or other component of the matrix. Since very few methods are absolutely specific, so the term **selectivity** is often used for this property and is defined as the degree to which a method can quantify the **analyte** (i.e. the micronutrient of interest) accurately in the presence of interferents. The smallest quantity of the analyte that can be distinguished from the background response or analytical noise by the method is known as the **limit of detection**.

**Sensitivity** is defined for the purpose of this manual as the degree of certainty that an analytical assay can differentiate between two very similar amounts of the analyte. The minimum amount of the analyte that can be quantitatively determined with suitable precision and accuracy is known as **limit of quantification**.

**Accuracy** is the capacity of the analytical method to determine the amount of the analyte as close as possible to the reality. Frequently, this property is checked by means of spiking the unfortified foods with known amounts of the nutrient (analyte) or analyzing Certified Reference Material (CRM). However, for fortified foods, CRMs are not readily available.

**Precision** is a general term for the variability among repeated tests under specified conditions. Two types of precision have been found necessary for describing the variability of a test method: 1) within-run variation also known as **repeatability**, and 2) between-run variation also named as **reproducibility**.

**Ruggedness** defines the degree to which the same method produces the same results in different laboratories and with different laboratory technicians. This is an important parameter to consider when an analytical method has sufficient reliability. Laboratory proficiency testing that compares results from different laboratory for the same samples is a practical way to confirm the ruggedness of a method.

---


II. METHODS FOR DETERMINING IODINE IN SALT

Methods for determining iodate or iodide in edible salt are very specific. Both methods are based on the generation of iodine and triiodide, which in turn react with starch to form a dark blue complex. When iodide is the fortificant compound, bromine water is added to transform all the iodide into iodate and, from that point, the assay follows the same reactions as with iodate. The methods presented in this manual are based on the same reaction, except that the final determination step varies. In the qualitative methods, the intensity of the blue color provides a general approximation of the amount of iodine in the solution, but many factors such as alkalinity of the salt and aging of the starch solution...
may modify the intensity, tonality and stability of the color. Furthermore, the reaction is generally made with a very small amount of the sample (such as in the case with the UNICEF kits), causing that the accuracy and precision of the method be unsatisfactory. Therefore, the qualitative methods (i.e. kits) should be used only for detecting the presence of iodine.

Titration with sodium thiosulfate is the most common method used to quantify iodine. Other instruments such as the Chinese Checker, a field spectrophotometer identified as UMS Instant Iodine Tester, may be used but they are less accurate and sensitive than the titrimetric assay.

Table 3 summarizes the properties of the iodine methods.

**Table 3. Comparison of performance parameters for methods to determine iodine in salt**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Qualitative test (UNICEF's kit)</th>
<th>Visible Spectrophotometry</th>
<th>Titrimetric method</th>
</tr>
</thead>
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<td>US$2.00</td>
<td>US$3.50</td>
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<td>-</td>
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<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Precision</td>
<td>+</td>
<td>↔</td>
<td>+++</td>
</tr>
</tbody>
</table>

(Footnotes)


3 Sensitivity in epidemiology has a different meaning, and it refers to the property of methods to respond to the parameter of interest.
(B). PROCEDURES FOR DETERMINING IODINE IN SALT

I. QUANTITATIVE TITRIMETRIC METHOD FOR DETERMINING IODINE FROM IODATE IN SALT

A. References


B. Principle

Most salts are fortified with potassium iodate (KIO$_3$). To determine the concentration of the added iodine as iodate, the salt is dissolved in slightly acidic solution to which excess potassium iodide (KI) is added. The iodate from the salt reacts with iodide (I$^-$) to form iodine (I$_2$) and triiodide (I$_3^-$), which is very soluble in water, and the solution turns yellowish. When a starch solution is added, a blue colored complex between triiodide and starch is formed. The amount of iodine in solution is determined by a colorimetric titration with a standard thiosulfate solution, which removes the iodine and as result the blue color disappears. The end point is visually determined by the disappearance of the blue color from solution when no more iodine is present.

The related chemical equations at various steps are as follows:

\[
\begin{align*}
\text{Formation of iodine from the iodate in salt solution} \\
\quad 1\text{O}_3^- (aq) + 5\text{I}^- (aq) + 6\text{H}_3\text{O}^+ (aq) & \rightarrow 3\text{I}_2 (aq) + 9\text{H}_2\text{O} \quad (1)
\end{align*}
\]

\[
\begin{align*}
\text{Formation of triiodide and blue complex with starch} \\
\quad \text{I}_2 (aq) + \text{I}^- \leftrightarrow \text{I}_3^- (aq) + \text{starch} & \rightarrow \text{Blue Complex} \quad (2)
\end{align*}
\]

\[
\begin{align*}
\text{Reduction of iodine with thiosulfate} \\
\quad \text{I}_3^- (aq) + \text{starch} + 2\text{S}_2\text{O}_3^{2-} (aq) & \rightarrow \text{S}_4\text{O}_6^{2-} (aq) + 3\text{I}^- (aq) \quad (3)
\end{align*}
\]

\[(\text{Blue solution}) \quad (\text{Colorless})\]

The overall reaction is:

\[
\begin{align*}
\quad 1\text{O}_3^- (aq) + 5\text{I}^- (aq) + 6\text{H}_3\text{O}^+ (aq) + 6\text{S}_2\text{O}_3^{2-} (aq) & \rightarrow 3\text{S}_4\text{O}_6^{2-} (aq) + 6\text{I}^- (aq) \quad (4)
\end{align*}
\]

The overall reaction implies that one equivalent of iodate (IO$_3^-$) reacts with 6 equivalents of thiosulfate. Therefore, in terms of iodate/iodine weight, one equivalent of thiosulfate means 35.667 grams of potassium iodate (FW KIO$_3$/6 = 214/6 = 35.667), or 21.222 grams of iodine, knowing that potassium iodate contains 59.5% iodine (35.667 x 0.595 = 21.222).

C. Critical Points and Cautions

The starch solution should be freshly prepared because it is easily destroyed by microorganisms. In any case, each time that the method is used, a control sample of iodized salt with a known amount of iodine should be analyzed first to confirm its reliability.
D. Equipment and Materials

- Beaker (250-500 mL)
- Glass rods
- Graduate cylinder (50 mL)
- Graduated pipettes, 1 to 5 mL
- Burettes or graduated pipettes (to measure 10-50 mL)
- 50mL pipette
- Containers calibrated by volume to weigh approximately 10 g of salt.
- Volumetric flasks 100 mL, 250 mL

E. Reagents

0.005 N-Sodium Thiosulfate Solution: Dissolve 1.24 g Na₂S₂O₃.5H₂O (FW= 248) in one liter of distilled water and store in a cool dry place. Solution is stable for 1 month. This amount is sufficient for about 200 samples.

2 N-Sulfuric Acid Solution: Using concentrated sulfuric acid, slowly add 60 mL to 900 mL of distilled water and mix. The solution is cooled down and made up to one liter. This amount is sufficient for about 1,000 samples.

10% Potassium Iodide Solution: Dissolve 100 g of potassium iodide in water and make up to one liter. Store in a cool dark place. This solution is stable for 6 months provided there is no color change. This amount is sufficient for about 200 samples.

Starch Solution: Weigh 1 gram of soluble starch into a 100 mL beaker and add 10 mL of water, heat to dissolve.

The thiosulfate solution can be standardized by titrating with a standard 0.005N-potassium iodate solution. This standard solution is prepared from a 0.5 N solutions made by dissolving 4.4585 g analytical grade KIO₃ in water and making up to 250mL. The 0.5 N solution is diluted 100-fold by taking 2.5 mL and diluting to 250 mL with distilled water. Normality of the thiosulfate solution = [volume KIO₃ (sol)/ volume thiosulfate (sol)]/ x Normality of iodate solution (0.005 N)

Prepare a saturated solution of sodium chloride dissolving NaCl in 80 mL of distilled water, heat up the solution until no more NaCl dissolves. Cool the solution and add to the dissolved starch, and make up to 100 mL. Store in a cool dark place. This amount is sufficient for about 50 samples. Prepare the starch solution every day. The saturated NaCl solution is stable for 12 months.

(Footnotes)

1 The thiosulfate solution can be standardized by titrating with a standard 0.005N-potassium iodate solution. This standard solution is prepared from a 0.5 N solutions made by dissolving 4.4585 g analytical grade KIO₃ in water and making up to 250mL. The 0.5 N solution is diluted 100-fold by taking 2.5 mL and diluting to 250 mL with distilled water. Normality of the thiosulfate solution = [volume KIO₃ (sol)/ volume thiosulfate (sol)]/ x Normality of iodate solution (0.005 N)

F. Procedure

a. Solubilization of the salt sample

1. Mix well the sample of salt and weigh accurately 50 gₖ and dissolve in a 250 mL beaker. Transfer to 250 ml volumetric flask and fill to the mark.

2. Transfer 50 mL of the salt solution using a 50 mL pipette to a 200 mL Erlenmeyer flask
3. Using a graduated pipette, add 1 mL of the 2-N sulfuric acid to the salt solution and mix thoroughly.
4. Add 5 mL of the 10% potassium iodide solution using a measuring cylinder or a pipette. If iodine is present a yellowish solution is formed.
5. Cover the flask and put in the dark or in a cupboard for 10 minutes.

Some procedures specify 10 grams of salt, but although this amount may be useful for highly refined salts, it is insufficient for raw and unrefined salts. In order to improve accuracy and precision of the method, at least 50 grams of coarse salt should be solubilized.

b. Titrating iodine in the salt solution

6. Fill the 50 mL burette with the thiosulfate solution in readiness for titration.
7. Titrate the iodine solution in the flask with the thiosulfate and stop the titration when the dark color of the solution turns to pale yellow. Agitate the salt solution continuously.
8. Add 2 mL of the starch solution and the solution should turn blue. Mix thoroughly.
9. Resume titration with thiosulfate until the blue color disappears. Agitate the salt solution continuously and gently.
10. Record the volume from the burette or serologic pipette as accurately as possible to the nearest 0.1 mL.

E. Calculations

1. The amount of potassium iodate in the salt is determined using the following equation.

   \[
   I \text{ (mg / kg)} = \frac{N \cdot \text{Vol Na}_2\text{S}_2\text{O}_3 (eq / L) \times Vol \text{ Na}_2\text{S}_2\text{O}_3 (mL) \times 21.222 \text{ (g / eq L)} \times \text{Initial volume (mL)}}{w_{salt} (kg) \times \text{Used volume (mL)}}
   \]

   If the procedure is strictly followed, the prior equation can be simplified to:

   \[
   I \text{ (mg / kg)} = \frac{0.005N \cdot \text{Vol Na}_2\text{S}_2\text{O}_3 \times \text{Vol Na}_2\text{S}_2\text{O}_3 (mL) \times 21.222 \text{ (g / eq L)} \times 250 (mL)}}{0.05 \text{ kg} \times 50 (mL)}
   \]

   The final equation is as follows:

   \[
   I \text{ (mg / kg)} = 10.61 \times \text{Vol Na}_2\text{S}_2\text{O}_3 (mL)
   \]
Chart 1 presents the equivalences between volume of the thiosulfate solution used and the amount of iodine in salt, under those conditions.

**CHART 1**

**CONVERSION CHART FOR IODINE IN FORTIFIED SALT (PARTS PER MILLION)**

Salt fortified with Iodate or Iodide

<table>
<thead>
<tr>
<th>Volume Thiosulfate (mL)</th>
<th>Iodine (ppm)</th>
<th>Volume Thiosulfate (mL)</th>
<th>Iodine (ppm)</th>
<th>Volume Thiosulfate (mL)</th>
<th>Iodine (ppm)</th>
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II. QUANTITATIVE TITRIMETRIC METHOD FOR DETERMINING IODINE FROM IODIDE IN SALT

A. Principle
The method for the determination of iodide in salt is similar to the methods for iodate determination except that it is preceded by the oxidation of iodide to iodate using bromine water. Excess bromine water is added to the iodide solution and any excess of bromine is destroyed using sodium sulfite and phenol solutions.

B. Reagents
In addition to the reagents listed in the method for determination of iodine from iodate above, the following are also necessary:

- **Saturated Bromine Water:** The approximate concentration in mg/L is determined by reaction of a known volume of the solution with excess KI solution. The released iodine which corresponds to the bromine in solution is titrated using standard 0.1N thiosulfate solution.

- **Procedure:** Add 5mL of 10% KI and 5mL of dilute sulfuric acid to a conical flask. Add the bromine solution from a burette and titrate the iodine generated using starch as an indicator. 1 mL of the 0.1N Na$_2$S$_2$O$_3$ = 8mg of bromine = 12.7mg of iodine.

- **Sodium sulfite, 1 % (m/v)** Dissolve 1 g of Na$_2$SO$_3$ and dissolve in distilled water and dilute to 100 mL water.

- **Methyl orange —** Dissolve 0.01 g methyl orange in water and dilute to 100 mL.

- **Phenol solution 5 % (m/v)** dissolves 5g of phenol in water and dilute to 100 mL.

An alternative method is using specific electrodes for iodide.

C. Procedure

a. Oxidation of iodide to iodate

1. Mix well the sample of salt and weigh accurately **50 g** of salt and dissolve in a 250 mL beaker. Transfer to a 250 ml volumetric flask and make up to the 250 mL mark with distilled water.
2. Transfer 50 mL of the salt solution using a pipette to a 200 mL Erlenmeyer flask. Add 6 drops of methyl orange (the solution turns pale orange). Neutralize with 2-N sulfuric acid, until a pink color appears.

3. Add bromine water drop-wise from a burette in a quantity equivalent to 10 mg of bromine (about 0.5 mL bromine water). The solution changes to yellow.

4. Allow the solution to stand for a few minutes and add sodium sulfite solution-1% drop-wise while mixing to destroy most of bromine, until solution turns pale yellow.

5. Wash down the neck and sides of the flask with water and complete the destruction of bromine by adding 1 or 2 drops of phenol solution-5%. The solution turns clear.

b. Determination of iodine from the iodate formed

6. Using a graduated pipette, add 1 mL of the 2-N sulfuric acid to the salt solution and mix thoroughly.

7. Add 5 mL of the 10% potassium iodide solution using a measuring cylinder or a pipette. If iodine is present a yellow solution is formed.

8. Cover the flask and put in the dark or in a cupboard for 10 minutes.

9. Follow with the titration step as explained for the determination of iodine from iodate (section F.b).

10. Carry out a blank determination of the reagents and make one or more control determinations using 100 mL of sodium chloride solution to which has been added appropriate quantity of potassium iodide control solution.
III. QUANTITATIVE METHODS FOR THE DETERMINATION OF IODINE FROM IODATE IN SALT USING THE UMS INSTANT IODINE TESTER

A. Reference

B. Principle
The Instant Iodine Tester is similar to a spectrophotometer using a single wavelength. A calibrated color glass is used instead of standard solution. It is suitable for use in the field of iodized salt manufacturing, checking compliance at the importation sites, and an alternative method to titration in a laboratory. However, it is important to point out that because the formation of the blue color may vary depending on the salt alkalinity as well as the age of the starch solution, this method has lower accuracy and precision than the titration method.

The principle of this method is the same as the titrimetric method to determine iodine in salt. Iodine in the salt sample is dissolved in a sulfuric acid solution and then a solution of potassium iodide (KI) and starch is added. The iodate from salt reacts with iodide (I⁻) to form iodine (I₂) and triiodide (I₃⁻), which is very soluble in water. A yellow color is formed first and, when starch solution is added, then a blue colored complex is formed between starch and triiodide.

The absorbance of this blue compound is read in the UMS which is proportional to the amount of iodine in the sample.

C. Critical points and cautions
The equipment must be warm up for ten minutes prior to be used. If it has not been used for a long time, let it warm up longer. Before reading the samples, be sure to keep the zero adjust controls and the standard adjust control in line with the marks on the surface of the UMS calibration keys.  

A normal visible spectrophotometer can be used instead of the UMS. In this case, the wavelength for the reading is the one with the highest absorbance by the iodine-starch solution. This wavelength is easily determined by means of running the absorbance spectrum of the solution.

Install the UMS in a dry place with good ventilation and avoid moist environment. Clean the salt and dust on instrument after use. Keep away from moist and damp environment while testing takes place.

D. Equipment and materials
- Analytical balance + 0.0001 g
- Dessicator
- UMS Instant Iodine Tester
- Plastic cuvettes for reading in the UMS
- Pasteur pipettes
- Beakers 50, 100, 250, 600 mL
- Erlenmeyer 50, 100, 250 mL
- Volumetric flasks 100, 200 mL
E. Reagents

a. Potassium iodate solution-1000 mg Iodine/L \((\text{KIO}_3, 99.7\%, \text{and 59.5\% iodine, FW 214.00})\). Weigh 0.8432 g KIO\(_3\) which is dried at 100-110°C for 3 hours and dissolve it in 500 mL distilled water (Calculations: 0.8432 g KIO\(_3\) x 0.997 x 0.595 as iodine = 0.5 g Iodine/0.5 L = 1 g Iodine/L = 1,000 mg Iodine/L).

b. Sodium chloride standard solution – 20% containing iodine at 10 µg/mL. Take 5 mL KIO\(_3\) standard solution and add 100 g NaCl (NaCl, 99.5%, FW 58.44) and 0.5 g Na\(_2\)CO\(_3\), add distilled water to mark 500 mL, and shake the solution thoroughly. It can be used for six months.

c. Starch-KI solution. (KI 99.5%, FW 166.01). Weigh 2 g of soluble starch, add distilled water and stir thoroughly. Transfer this into 200 mL boiling water and keep on boiling for two minutes, then cool, add 1.0 g KI and 40 g K\(_2\)HPO\(_4\)·3H\(_2\)O. This solution can be stored in a bottle up to three months.

d. Sulfuric acid solution-1 M \((\text{H}_2\text{SO}_4, 95-98\%, \text{FW 98.08})\). Pour carefully 11 mL concentrated sulfuric acid into a beaker containing about 190 mL distilled water and mix thoroughly. Store in a cool, dark place, separated from bases.

F. Procedure

a. Preparation of standard solution to calibrate the UMS

1. Use the colored glass to calibrate the UMS or prepare the standard solution.

2. Transfer 5.0 mL iodine standard solution (10 µg/mL) into a 50 mL tube, add 2 mL KI-starch solution and 2 mL sulfuric acid solution-1M.

3. Mix the solution thoroughly, and add distilled water to make 50 mL.

4. Shake well. This solution is equal to the concentration of 50 mg I/kg salt, because in the method 50 g of the salt samples are diluted with 250 mL of water, and from this solution 5 mL are used for the reaction, such as it is the case with the standards.

b. Preparation of sample solution

5. Weigh 50 g well-mixed iodized salt in a 500 mL beaker and add 250 mL distilled water.

6. Dissolve the salt sample completely.

7. Take 5 mL sample solution and add 2 mL KI-starch solution and 2 mL H\(_2\)SO\(_4\)-1M.

8. Mix the solution and add distilled water to make 50 mL.
c. Calibration of the UMS

9. Turn on the power of UMS and warm up it for 10 minutes.

10. Adjust the zero and standard auto calibration buttons as to keep them aligned to the marks on the surface of the UMS calibration key.

The original instruction of the method suggest 10 grams of salt diluted in 100 mL, but this amount of salt is too small to provide reliable results, especially for coarse salts.

11. Fill up the cell with distilled water and place it into the cell chamber and close the cover. The clear surface of the cell must be placed in line with the output of the light bulb left to right, as well as keep the clear surface of the glass close to the head of arrow of the sample cell chamber.

12. Turn zero adjustment blue calibration key (left) until it shows on readout digital. Turn on the blue calibration key to adjust integer numbers to zero. Use the white calibration key (right) to adjust the decimal numbers to zero.

13. As soon as zero is reached in the LCD readout, keep the zero calibration constant. **Note:** Do not touch the zero calibration anymore.

14. Insert the standard solution or colored glass into the cell chamber and turn it to attain 50 at LCD readout by adjusting (the left) blue calibration key to adjust integer numbers to zero and (the right) white calibration key to adjust decimal to zero in the LCD readout. As soon as zero is reached at LCD readout, keep the standard calibration constant.

15. Take out the standard solution or calibration glass, insert the sample solution into the cell chamber and close the cover. The iodine concentration (mg/kg) is clearly readout in the UMS. Write down the content of iodine in the sample.

16. Keep on reading the content of iodine in different sample simultaneously one after another.

**Note:** It is not necessary to run the zero calibration and standard calibration between samples, except when the UMS is turned off.

G. Interpretation of results

Write down the reading in the LCD readout. The result is iodine concentration in mg/kg.
ANNEX 1. INTERNAL QUALITY CONTROL OF METHODS

A. References

B. Principle
Guaranteeing reliability of results reported by a laboratory is one of the major challenges laboratories face everyday. The implementation of an internal quality control program to monitor the performance of methods and results of measurements to decide whether they are reliable enough to be informed is fundamental in the analytical laboratory.

This chapter has been included as a guide to implement an internal quality control program in the laboratory for the analytical methods. The steps here refer to sugar, but they can be also adapted to sugar premix, oil, flours and salt.

The program comprises preparing a control sample of the fortified food (in-house reference material) that is run along the samples received in the laboratory. The average micronutrient concentration and its standard deviation are determined for the control sample. A Shewhart chart is built based on these parameters and control limits are plotted.

These control limits are numeric limits within which the control sample values should fall in every run. The control sample values are assessed every time samples are analyzed to verify that the analytical process is within control. Inhouse control material is used here for two reasons: 1) Certified Reference Materials (CRM’s) for fortified foods are not available, except for some flours; and 2) analyzing the CRM in every run would be too expensive.

C. Procedure
1. Take about 15 kg unfortified sugar and fortify it with 10-15 g vitamin A premix to obtain a retinol level between 10-15 mg/kg. Homogenize the sugar. This amount of control sample should last 3-4 months.
2. Determine the retinol content in the control sample until 20 values are obtained. The analysis should be performed by different analysts preferably.
3. **Control parameters**: Calculate the arithmetic mean, the standard deviation and the coefficient of variation.

4. Build a Shewhart chart (Levy-Jennings) setting the days or runs (1 to 20) in which the control will be used along the x-axis. The y-axis corresponds to the retinol concentration expressed in mg/kg.

5. Plot the values for the mean, mean ± SD (normal limits), mean ± 2 SD (caution limits) and the mean ± 3 SD (action or rejection limits). It is recommended to use colors to differentiate the limits to facilitate the visual interpretation of results. Then, green is assigned to the retinol mean, blue to the normal limits, orange to the caution limits and red to the action or rejection limits. Table F-1 presents an example of the table to record results and the chart obtained.

6. Every time sugar samples are analyzed, take a portion of the control and determine its retinol content using the same method applied to the samples.

7. Calculate the retinol concentration in the control and plot the results in the chart.

**D. Interpretation**

The analytical system is out of control when:

1. The control value falls outside the action limits (mean ± 3 SD). The run is rejected and samples should be analyzed again.

2. The control values from the current and previous runs fall outside the caution limits (mean ± 2 SD), but within the action limits.

3. Nine consecutive control values fall on the same side of the mean. In the case of retinol this can happen because of the decrease of retinol concentration in the control. Therefore, determine again the control limits or change the control.

4. If none of the conditions mentioned above are found, the run is accepted.
# TABLE 1

**INTERNAL QUALITY CONTROL OF THE SPECTROPHOTOMETRIC METHOD FOR DETERMINING RETINOL IN SUGAR FORTIFIED WITH VITAMIN A**

<table>
<thead>
<tr>
<th>Control:</th>
<th>Sugar</th>
<th>Method:</th>
<th>Spectrophotometric determination of retinol in sugar</th>
</tr>
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<tbody>
<tr>
<td>Analyte:</td>
<td>Retinol</td>
<td>Expressed in:</td>
<td>mg/kg</td>
</tr>
<tr>
<td>Year:</td>
<td>2007</td>
<td></td>
<td></td>
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<table>
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<tr>
<th>Date</th>
<th>R-1</th>
<th>R-2</th>
<th>Sum</th>
<th>Mean</th>
<th>S.D.</th>
<th>C.V.</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**INTERNAL QUALITY CONTROL CHART**

![Graph showing Retinol concentration over time]
## ANNEX 2. SUPPLIERS FOR LABORATORY EQUIPMENT AND REAGENTS

<table>
<thead>
<tr>
<th>Number</th>
<th>Supplier Name</th>
<th>Address</th>
<th>Phone Numbers</th>
<th>Websites</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sigma-Aldrich</td>
<td>3050 Spruce St., St. Louis, MO 63103</td>
<td>Tel: (314) 771-5765; Fax: (314) 286-7817</td>
<td><a href="http://www.sigmaaldrich.com">www.sigmaaldrich.com</a></td>
</tr>
<tr>
<td>2.</td>
<td>BASF-Denmark</td>
<td>Health &amp; Nutrition, Malmparken 5</td>
<td>Tel: (45) 447-30100; Fax: (45) 447-30101</td>
<td><a href="http://www.corporate.basf.com">www.corporate.basf.com</a></td>
</tr>
<tr>
<td>3.</td>
<td>Taylor Scientific</td>
<td>950 Hanley Industrial Court, St Louis, MO 63144</td>
<td>USA</td>
<td>Tel: +1-314-962-5555; Fax: +1-314-962-9382; Toll Free: 800-727-0467</td>
</tr>
<tr>
<td>4.</td>
<td>Beckman Coulter, Inc.</td>
<td>4300 N. Harbor Boulevard, Fullerton, CA 92634-3100</td>
<td>PO Box 3100</td>
<td>Tel: (800) 742-2345; Fax: (800) 643-4366</td>
</tr>
<tr>
<td>5.</td>
<td>Fisher Scientific</td>
<td>2000 Park Lane, Pittsburgh, PA 15275</td>
<td>USA</td>
<td>Tel: (800) 766-7000; Fax: (800) 926-1166</td>
</tr>
<tr>
<td>6.</td>
<td>DSM Human Nutrition &amp; Health</td>
<td>PO Box 3255, Building 241, CH-4002, Basel, Switzerland</td>
<td>Tel: 41(61)688-3333; Fax: 41(61) 688-3330</td>
<td><a href="http://www.dsm.com">www.dsm.com</a></td>
</tr>
<tr>
<td>7.</td>
<td>Mallinckrodt Baker, Inc.</td>
<td>222 Red School Lane, Phillipsburg, NJ 08865</td>
<td>USA</td>
<td>Tel: (908)859-2151; Fax: (908) 859-9318</td>
</tr>
<tr>
<td>8.</td>
<td>Millipore</td>
<td>290 Concord Rd, Billerica, MA 01821</td>
<td>USA</td>
<td>Tel: (978) 715-4321</td>
</tr>
<tr>
<td>9.</td>
<td>National Institute of Standards and Technology (NIST)</td>
<td>Standard Reference Materials Program</td>
<td>Tel: (301) 975-6776; Fax: (301) 948-3730</td>
<td><a href="http://www.nist.gov">www.nist.gov</a></td>
</tr>
<tr>
<td>10.</td>
<td>Perkin Elmer, Life and Analytical Sciences, Inc.</td>
<td>710 Bridgeport Ave, Shelton, CT 06484-4794</td>
<td>USA</td>
<td>Tel: (203) 925-4600; Fax: (203) 944-4904</td>
</tr>
<tr>
<td>11.</td>
<td>Merck KGaA</td>
<td>Frankfurter St. 250, 6293 Darmstadt, Germany</td>
<td>USA</td>
<td>Tel: (49) 6151-72-0; Fax: (49) 6151-72-2000</td>
</tr>
<tr>
<td>12.</td>
<td>EMD Chemicals Inc.</td>
<td>Analytics &amp; Reagents Division, Life Science Prod. Division</td>
<td>Gibbstown, NJ 08027, USA</td>
<td>Tel: (856) 423-6300; Fax: (856) 423-4389</td>
</tr>
<tr>
<td>13.</td>
<td>Cole Palmer Instruments Co</td>
<td>635 East Bunker Court, Vernon Hills, IL 60061-1844, USA</td>
<td>Tel: (847) 323-4340</td>
<td><a href="http://www.coleparmer.com">www.coleparmer.com</a></td>
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The publication of this manual is made possible by the generous support of the American people through the US Agency for International Development (USAID), through the Academy for Educational Development, A2Z: The USAID Micronutrient and Child Blindness Project (GHS-A-00-05-00012) and the East, Central and Southern African Health Community (ECSA). The content of this document is the responsibility of the authors and does not necessarily reflect the opinion of USAID or the government of the United States.